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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Douglas A. Treco et al. Art Unit : 1646
Serial No. : 09/716,166 Examiner : D. Jiang
Filed : November 17, 2000
Title : CONSTRUCTS AND CELLS FOR PRODUCTION OF SMALL PEPTIDES

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1.132 OF MICHAEL CONCINO, Ph.D..

I, Michael Concino, Ph.D., pursuant to 37 C.F.R. § 1.132, declare the following:

1. I am currently employed by Shire Pharmaceuticals, which acquired Transkaryotic Therapies Inc., as the Senior Director of Protein Chemistry. I am also an inventor on the above-referenced application. My Curriculum Vitae is attached.

2. I have read the application and the Office Action dated April 6, 2005, which has rejected claims 1-6, 8-14, 17, 19, 21-35, 37-46, 83-93 as allegedly being obvious.

3. I assisted in the design, execution and analysis of the experiments described herein.

4. The constructs of the invention resulted in surprisingly high levels of secretion from non-endocrine cells.

Warren et al. (Cell 39:547-555 (1984)) discuss expression and secretion of somatostatin in COS cells when somatostatin is associated with its naturally occurring prepro region. In that reference, Warren et al. report that using sensitive detection methods such as high performance liquid chromatography (HPLC) relatively low levels of secreted somatostatin can be detected. Warren et al. further provide that somatostatin secretion may not have been detected if SDS-

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I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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PAGE was used for detection. See page 553, paragraph abridging columns 1 and 2 of Warren et al. Thus, based upon teachings such as Warren et al., it would be expected that even if GLP-1 was secreted by non-endocrine cells when associated with the prepro-region of somatostatin, it would be at fairly low levels.

5. Experiments were conducted to try to obtain secretion of GLP-1 from non-endocrine cells using various combinations of pre and/or pro regions, including the pre and pro regions naturally associated with GLP-1. With the exception of GLP-1 associated with the prepro region of somatostatin, none of the various pre and/or pro region combinations resulted in detectable secretion of GLP-1 from fibroblasts.

As indicated above, even when GLP-1 was introduced into non-endocrine cells with its naturally associated prepro region, no GLP-1 secretion was detected. Specifically, a vector containing nucleic acids encoding the signal peptide and propeptide from glucagon as well as GLP-1 was transfected into fibroblasts. Using pulse chase detection and radioimmunoassay (RIA) quantification methods, no secretion of GLP-1 from the fibroblasts was detected. From our experiments, we concluded that GLP-1 with its naturally associated prepro region was being degraded intracellularly.

Other combinations of pre and/or pro regions were tested for their ability to cause GLP-1 secretion. The pre and pro regions tested included: hGH signal peptide, factor IX prepro region and truncations thereof, SNV signal peptide, protein C signal peptide and truncations thereof, and IGF-1 prepro region. None of these constructs resulted in GLP-1 secretion from fibroblasts.

Two vectors containing nucleic acids encoding the prepro region of somatostatin and GLP-1 were constructed. In contrast to the results obtained with vectors containing GLP-1 with its own prepro region or any other pre and/or pro region tested, when the vectors containing nucleic acids encoding the prepro region of somatostatin as well as GLP-1 were transfected into fibroblasts, GLP-1 was secreted in active form at levels of 5-9.5 ng/1 x 10^6 cells/24 hours as detected by RIA. This was surprising.

In my opinion, it is not clear why only the somatostastin prepro region, and none of the other pre and/or pro regions tested (including GLP-1's own naturally occurring pre pro region),

could guide GLP-1 through the secretory pathway. Further, in my opinion, this result could not have been predicted from Warren et al., as this reference teaches that the prepro region of somatostatin can only cause low (sometimes undetectable) levels of secretion of somatostatin peptide naturally associated with the prepro region.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

DATE: April 4, 2006

Michael Concino, Ph.D.

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CURRICULUM VITAE

Michael F. Concino
297 Longhill Rd.
Bolton, MA 01740
(978) 779-9914
mconcino@comcast.net

EDUCATION:

1981	Ph.D., Graduate Group in Molecular Biology, University of Pennsylvania. Thesis title: "DNA uptake by *Haemophilus influenzae*; Detection and Isolation of the DNA receptor." Advisor: Sol H. Goodgal, Professor, Department of Microbiology
1977	B.S., Major: Biology York College of Pennsylvania Magna cum laude
1975	Summer Adjunct Student, Pennsylvania State University

Summary of Qualifications:

- 20 years of Biotechnology Industry experience in the area of Molecular Biology, Cell Biology, and Protein Biochemistry with a strong emphasis in Gene Expression.
- Experienced at managing scientists, goal setting, and budgeting for department resources. Friendly, focused and goal-oriented individual.

PROFESSIONAL EXPERIENCE:

July 2005 to Present	Shire Pharmaceuticals, Inc. Human Genetic Therapies, Cambridge, MA **Senior Director, Protein Expression and Purification Research** TKT, Inc. acquired by Shire
February 2005 to July 2005	Transkaryotic Therapies, Inc, Cambridge, MA **Senior Director, Protein Expression and Purification Research**

- The Company's Exploratory Research Department was merged with my Protein Chemistry Department expanding the scope of responsibilities.

March 2003 to February 2005	Transkaryotic Therapies, Inc, Cambridge, MA **Senior Director, Protein Chemistry**

- At the time of reorganization at TKT, I was chosen to create a new research department whose purpose was to provide protein purification, analytical, and assay development support to Research.

This position reports to the Vice President of Research. Research was revamped to provide a more focused goal-oriented approach with relatively limited turnaround times and "proof of principle" experiments as objectives.

- N-acetylgalactosamine-6-sulfatase
- Acid alpha glucosidase
- Iduronate-2-sulfatase
- Tissue non-specific Alkaline Phosphatase
- Alpha-galactosidae
- Beta-galactosidase
- Heparan-N-sulfatase
- Ornithine Transcarbamylase

December 1998 to March 2003 — Transkaryotic Therapies, Inc., Cambridge, MA
Senior Director of Therapeutics

- Project Leader for Diabetes Gene Therapy Program. The approach taken required development of a proprietary process for the production and *in vivo* delivery of an insulinotropic peptide therapeutic. Responsibilities include design, construction, and evaluation of mammalian expression plasmids, development of cell-based assays, immunoassays, purification and characterization of the therapeutic peptide, and pre-clinical animal testing. Demonstrated long-term delivery and efficacy in *db/db* diabetic mice.

- Developed recombinant human cell lines producing therapeutic proteins for treatment of Pompe, and Sanfillipo B diseases.

- Additional responsibilities included the myoblast Gene Therapy Group that focused on developing a protocol for Hemophilia B. With the addition of these resources, I was responsible for nearly all Gene Therapy research at the Company.

- Played leadership role in the continuing Hemophilia A Gene Therapy research activities as the project moved to emphasize animal testing.

- Responsible for up to 15 scientists and coordinated interdepartmental efforts for design, development, and evaluation in animals, of critical second-generation component for the next Phase of the Hemophilia A clinical trial.

- Responsible for the Company's research assay development effort by developing and providing antibody-based assays and reagents to support Company projects. Developed Host Cell Protein Assay for protein-based drug development

May 1992 to December 1998 — Transkaryotic Therapies, Inc., Cambridge, MA
Director of Therapeutics

- Responsible for design, construction, and evaluation of expression plasmids to support the Company's Hemophilia A Gene Therapy Program. During this time my group constructed over 600 human Factor VIII expression plasmids and evaluated most of these in primary human fibroblasts.

- Responsible for design, development, and implementation of gene therapy protocols for treatment of Hemophilia B and Hypercholesterolemia.

- Established the Factor IX program, directed and coordinated pre-clinical studies. Supervised a group of molecular biologists and tissue culture specialists focusing on mammalian expression vector development and evaluation. Conducted research focusing on transcriptional and translational mechanisms, RNA stability, and position effects as they relate to vector development. Over 200 Factor IX expression plasmids were designed, constructed, and tested using a stable transfection assay.

- Established the Company's protein biochemistry effort, hired and supervised biochemists, purified and characterized three therapeutic proteins expressed in heterologous systems. Developed ELISA immunoassays as required for support of gene therapy programs.

January 1991 to May 1992 — Procept, Inc., Cambridge, MA
Director of Protein Expression

- Responsible for genetic engineering of receptor genes, their expression and the production of these recombinant proteins in mammalian and prokaryotic cells.

- Developed soluble forms of lymphoid T-Cell receptor proteins.

- Developed mammalian expression vectors for expression and production of recombinant proteins.

- Developed transfected T Cell lines with altered antigen specificity.

- Developed Chinese hamster ovary cell lines expressing recombinant proteins used for production. Developed assays for monitoring and quantitating production of recombinant proteins.

- Produced gram scale quantities of recombinant proteins and monoclonal antibodies for Company and collaborative requirements. Operate along with senior scientific staff for program planning and company operation.

- Chairman of the Recombinant DNA Committee and Radiation Safety Officer.

December 1988 to January 1991 — Procept, Inc., Cambridge, MA
Director of Molecular Biology

- Played major role in setting up facility and initial effort for Company research and development.

- Established Molecular Biology group and staffed to the level of three junior and five senior scientists.

- Instrumental in planning and carrying out therapeutic development program. Involved with other senior managers in planning and conducting preclinical animal studies. Participated in evaluating potential projects.

- Responsible for company Molecular Biology effort that entailed engineering of receptor genes for therapeutic development and their expression in mammalian and bacterial systems.

- Established method for bench-top scale mammalian cell production of recombinant proteins and antibodies. Provided recombinant DNA and immuno-based biologicals for all in-house use.

- Established in-house radiation safety policy. Obtained license from the Nuclear Regulatory Commission and acted as Radiation Safety Officer.

- Established recombinant DNA policy and acted as Chairman of Recombinant DNA Committee.

December 1988 to November 1989

Procept, Inc., Cambridge, MA
Acting Director of Immunology

- Supervised one junior and one senior scientist for set up of immunology-based bioassays used for in vitro evaluation of recombinant proteins.

June 1988 to December 1988

T Cell Sciences, Inc., Cambridge, MA
Senior Scientist III, Manager, Protein Expression

- Directed R & D effort for all in-house recombinant DNA expression-related programs. Direct supervision of group consisting of four Ph.D.-level and six M.S../B.S.- level molecular biologists and biochemists.

- Initiated and conducted research collaborations with academic research laboratories. Interacted and collaborated with academic research laboratories. Interacted and collaborated with members of the scientific advisory board.

- Responsible for overseeing and reporting on scientific efforts for research contracts with pharmaceutical companies. Designed recombinant-based therapeutic proteins.

- Interfaced with and supported diagnostic R. & D. on ELISA kit development. Chairman of Recombinant DNA/Biosafety Committee.

November 1985 to June 1988

T Cell Sciences, Inc., Cambridge, MA
Senior Research Scientist II, Head, Protein Expression

- Established mammalian cell expression system currently used in house for production of modified/unmodified receptor proteins used in core technology development and diagnostic kits.

- Direct supervision of expression group consisting of four M.S./Ph.D.-level personnel and oversaw/coordinated protein biochemistry support consisting of three B.S./Ph.D.-level personnel.

- Project leader for core technology development and research contract programs. Brought research-level technology for scale-up of biologicals based on hollow fibers.

- Member of steering committee associated with pharmaceutical company-funded contract.

- Chairman of Recombinant DNA/Biosafety Committee. Contributed to invention disclosures and patent filings.

- Interacted and collaborated with members of the scientific advisory board and other members of the scientific community well known in the field of molecular immunology.

May 1984 to October 1985 — Damon Biotech, Inc., Needham, MA
Research Scientist in Recombinant Research Group

- Development of mammalian expression vectors for use in large-scale manufacturing of human therapeutic proteins.

September 1981 to May 1984 — Wistar Institute, Philadelphia, PA
Postdoctoral Fellow

September 1967 to September 1973 — Automotive Mechanic
Completed nine month Manpower Development Training Course in Auto Mechanics. Upon completion was employed by BMW car dealership as a mechanic. Completed periodic BMW factory training courses and worked through this period in the U.S. and in Scandinavia.

PATENTS

Awarded:

Patent No. 5,212,071. Nucleic Acids Encoding a Human C3b/C4b Receptor (CR1).

Patent No. 5,256,642. Compositions of Soluble Complement Receptor 1 (CR1) and a Thromboltic Agent, and the Methods of Use Thereof.

Patent No. 5,472,939. Method of Treating Complement Mediated Disorders.

Patent No. 5,817,789. Chimeric Proteins For Use in Transport of a Selected Substance into Cells.

Patent No. 5,856,297. Human C3b/C4b Receptor (CR1)

Patent No. 5,981,481. Human C3b/C4b Receptor (CR1)

Patent No. 6,027,921. Chimeric Proteins for Use in Transport of Selected Substance into Cells and DNA encoding Chimeric Proteins.

Patent No. 6,262,026. Chimeric Proteins for Use in Transport of Selected Substance into Cells.

Patent No. 6,316,604. Human C3b/C4b Receptor (CR1)

AWARDS AND HONORS

1974-1977	Dean's List, York College
1976	Danforth Fellowship Nominee
1977	Biology Departmental Honors
1975 and 1977	Who's Who in American Colleges and Universities
1976	Senior Honor Society
1976 and 1977	Ehrenfeld Award in Chemistry
1976-1977	American Chemical Society Certificate of Merit
1979-1981	Molecular and Cellular Biology Predoctoral Training Grant #5 T32 GM 07229-07
1/82-9/83	National Institutes of Health Wistar Institutional Postdoctoral Trainee, #CA 09171-08
9/83-4/84	National Institutes of Health Fellowship, #A1/GM 06774-01A1 BI

PUBLICATIONS/POSTERS/REPORTS

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Concino, M.F., M. Kahn, and S.H. Goodgal. 1979. A DNA binding particle isolated from a competence deficient mutant of *Haemophilus influenzae*. Fed. Proc. Abstract 3080.

Kahn, M., M.F. Concino, and S.H. Goodgal. 1979. The occurrence of DNA binding activity in vesicles produced by a *H. Parainfluenzae* mutant, defective in transformation. XI^{em} Congress Internationale de Biochemie.

Kahn, M., M.F. Concino, R. Gromokova, and S.H. Goodgal. 1979. DNA binding activity of vesicles produced by competence deficient mutants of *Haemophilus*. Biochem. Biophys. Res. Commun. 87: 764-772.

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Concino, M.F., R. Goldman, M. Caruthers, and R. Weinmann. 1983. Point mutations of the adenovirus major late promoter with different transcriptional efficiencies in vitro. J. Biol. Chem. 258: 8493-8496.

Weinmann, R., S. Ackerman, D. Bunick, M.F. Concino, and R. Zandemeni. 1983. *In vitro* transcription of adenovirus genes. In: Current Topics in Microbiology and Immunology. (Doerfler, W., ed.) Springer-Verlag, New York, pp. 125-145.

Concino, M.F. and R. Weinmann. 1983. The effect of single base changes on *in vitro* transcription. I.C.R.F. DNA Tumor Meeting, Cambridge, England.

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Weinmann, R., S.P. Shi, M.F. Concino, and R. Lee. 1987. Genetic and functional analysis of the adenovirus major late promoter. Annual Meeting- American Society for Virology. University of North Carolina, Chapel Hill, North Carolina.

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